IJMST

ISSN: 0974-5343
IJMST (2021), 10(1):1-7

DOI: http://doi.org/10.5281/zenodo.4767293

Oxidative stress parameters in atherosclerotic cardiovascular disease high and low risk score groups as indicators for acute myocardial infarction

Mathew John[1], Priyanka S[2], Joann B George[3], Evelyn Maria[4], Govindan Unni[5]
1-4 Jubilee Centre for Medical Research, Jubilee Mission Medical College & Research Institute, Thrissur, Kerala, INDIA
5 Department of Cardiology, Jubilee Mission Medical College & Research Institute, Thrissur,Kerala, INDIA
mathewjohn@jmmc.ac.in, priyankanageswar@gmail.com, joanngeorge97@gmail.com, evu1joemercy@gmail.com, unni.govindan@gmail.com

Abstract

Background

Coronary artery diseases (CAD) are the leading cause of morbidity and mortality worldwide. Oxidative stress is an important event associated with CAD and dictates the associated pathophysiology. The imbalance in pro oxidants and antioxidants occurs during myocardial infarction and can cause damage to myocardium. Hence, it is worth investigating the oxidative stress status in atherosclerotic high risk group which may lead to myocardial infarction. Further, the comparative investigation of oxidative stress parameters between NSTEMI and STEMI variants of myocardial infarction in ECG helps to identify the differential response of pro and antioxidants according to the extent of coronary artery occlusion.

Objective

To study the role of oxidative stress molecules as early indicators of myocardial infarction in atherosclerotic disease high risk factor group. To investigate the antioxidant enzymes had any differential activity between NSTEMI and STEMI variants of myocardial infarction which can be indicators for severity of coronary artery occlusion.

Materials and methods

The study is designed as a comparative analysis of oxidative stress parameters between atherosclerotic disease high risk score category and low risk score category and between NSTEMI and STEMI variants in ECG during myocardial infarction. The sample size was n=26 in each group. The oxidative stress parameters were assessed in blood plasma of participants according to established methods. The statistics was done in Microsoft excel and students t test was used for comparison between groups and p value less than 0.05 considered as significant.

Results

The study observed that the antioxidant parameters such as catalase and glutathione had lowered activity in ASCVD high risk group compared to low risk group and no significant change was observed in superoxide dismutase and thiobarbituric acid reacting substances. We also observed that both antioxidant enzymes, Catalase and SOD had significantly lowered activity in STEMI vs NSTEMI variants in ECG of myocardial infarction group.

Conclusion

The study concluded that antioxidant enzymes lowered during ASCVD high risk category could be used as early indicators for acute myocardial infarction.

Key words: oxidative stress, antioxidants, catalase, superoxide dismutase

Introduction

Coronary artery disease (CAD) is one of the serious health problems affecting worldwide and major cause of mortality. It is well known that CAD is characterised by atherosclerosis in coronary arteries and can be asymptomatic, whereas acute coronary syndrome (ACS) is presented with distinct symptoms such as unstable angina and frequently manifested with myocardial infarction (MI) [1, 2]. Acute Coronary syndrome (ACS) comprises, unstable angina, NSTEMI and STEMI. STEMI results from complete and prolonged occlusion of an epicardial coronary blood vessel. NSTEMI usually results from severe coronary artery narrowing, transient occlusion or microembolization of thrombus and deposition of atheromatous materials in blood vessels[3,

IJMST

ISSN: 0974-5343
IJMST (2021), 10(1):1-7

DOI: http://doi.org/10.5281/zenodo.4767293

4].During myocardial infarction (MI) there occurs extensive cardiac remodelling which contributes significantly to ventricular dysfunction. Post MI, oxidative stress is developed in both infarcted and non-infarcted myocardium [5]. There were evidences from related studies that oxidative stress contributed in several aspects of cardiac repair and remodelling following infarction that include cardiomyopathic apoptosis, inflammatory/ fibrogenic responses, and hypertrophy [6, 7]. Acute coronary syndrome encompasses a sequence of cardiac events, ranging from unstable angina to ST segment elevation (STEMI) myocardial infarction [8]. Acute myocardial infarction of NSTEMI and STEMI is a highly dynamic event which is associated with increased production of reactive oxygen species (ROS). The imbalance in redox status leads to oxidative stress. Myocardium experiences oxidative insults in all variants of cardiac diseases and the oxidative modified molecules such as lipids and proteins not only act as determinant of severity of tissue injury but may be useful in the diagnostic and prognostic indices as a biomarker[9]. Myocardium possesses strong defence mechanism to counter the oxidative challenge via antioxidant defence machinery in the form of superoxide dismutase (SOD), catalase, glutathione (GSH) and glutathione peroxidase.Excessive production of reactive oxygen species (ROS) is an important event which could mediate ischemic reperfusion injury associated with MI. Exposure to ROS during MI lead to activation of various antioxidant defence mechanism to neutralise the excess free radicals.

Materials and Methods

The study was done in accordance with Institutional ethics and research guidelines and approval from Institutional ethics committee. The study involved participants in the age between 40 and 65 years who had high or low score for ASCVD and other set who suffered an ST elevated myocardial infarction (MI) and non-ST elevated MI, admitted to the coronary care unit of tertiary care hospital. The MI was confirmed by symptoms of ischemia and elevation in cardiac biomarkers such as troponin T.The age matched healthy samples were collected from people who came for regular health check up. Patients with multiple organ failure and those with cardiomyopathy, severe valvular disease, atrial fibrillation, chronic kidney disease requiring hemodialysis were excluded from the study. The atherosclerotic cardiovascular disease (ASCVD) risk score was estimated by an application named ASCVD risk estimator plus. The variables that statistically merit inclusion in the risk assessment equations are age, total cholesterol, HDL-cholesterol, systolic blood pressure, diabetes mellitus and current smoking status [10]. ASCVD risk score estimating the 10 year risk of the first ASCVD event in a life time was used and 7.5% was the cut off. Those subjects with a score less than 7.5% was considered in the low risk category while those with a score equal to or greater than 7.5% was considered in the high risk category.

The blood was collected inheparinised tubes and centrifuged at 3000 rpm for 5 minutes. The plasma was separated and taken for analysis. The study involved assessment of both pro-oxidant and antioxidant parameters. The pro-oxidant parameter included for analysis werethiobarbituric acid reacting substances (TBARS) and antioxidants assayed was glutathione and antioxidant enzymes assayed were superoxide dismutase and catalase. All assays were performed according to standard protocol. Lipid peroxidation level

IJMST

ISSN: 0974-5343
IJMST (2021), 10(1):1-7

DOI: http://doi.org/10.5281/zenodo.4767293

was estimated by measurement of thiobarbituric acid reacting substances (TBARS) in plasma according to development of pink chromogen complex formed by reaction of TBARS in plasma with thiobarbituric acid according to Satoh et.al [11]. Protein carbonyl content in plasma was detected by dinitro phenyl derivatization assay by Levine et.al [12]. Catalase activity was assessed according to spectrophotometric method of Goth [13]. The assay is based on the ability of H_2O_2 to form a stable complex with ammonium molybdate measured at 405 nm. Superoxide dismutase (SOD) activity was done based on the principle of autooxidation of pyrogallol by superoxide anion according to Marklund et.al [14].Glutathione estimation was done by enzymatic recycling method using NADPH and di thionitro benzoic acid (DTNB)[15]. Oxidised protein detection was done by Western blot according to commercially available kit procedure from abcam.The three plasma samples of clinically homogenous nature were pooled from control, STEMI and NSTEMI groups and subjected to Western blot. Briefly, the samples were derivatized with 1XDNPH (di nitro phenyl hydrazine) solution followed by addition of neutralisation solution provided in the kit. The samples were subjected to SDS-PAGE followed by transfer on to the nitrocellulose membrane. After blocking the reaction using blocking buffer the samples were incubated with 1X primary anti-DNP antibody in buffer followed by incubation and washing using 1X PBST (Phosphate buffred saline in Tween 20). The samples were then incubated with 1X HRP (Horse radish peroxidase) conjugated secondary antibody in blocking buffer. The bands were visualised colorimetrically using DAB (3, 3^1 diamino benzidine) reaction by oxidation reaction with H_2O_2. .

Table 1

Table 1: Comparison of oxidative stress parameters between ASCVD low risk score and high risk score group with cut off as 7.5%

Parameter	ASCVD risk score < 7.5 %(Mean±SD)	ASCVD risk score > 7.5 % (Mean±SD)	p value (Mean±SD)
Plasma Catalase (KU/L)	145.846 ± 23.451	126.153 ± 42.545 *	P < 0.05, p = 0.043
Plasma GSH (mg/dL)	0.313 ± 0.121	0.241 ± 0.08 *	P < 0.05, p = 0.029
Plasma SOD (U/L)	188.5 ± 18.363	173.73 ± 39.674 ns	P > 0.05, p = 0.158
Plasma TBARS (µmol/L)	0.383 ± 0.175	0.432 ± 0.205 ns	P > 0.05, p = 0.365
Protein carbonyls (nmol/mg protein)	0.3944 ± 0.187	0.5373 ± 0.207	P > 0.05, P = 0.068

From table 1 it was observed that plasma catalase and glutathione had decreased levels in ASCVD high risk group significantly. The plasma SOD, TBARS and protein carbonyls did not alter significantly across ASCVD group.

IJMST

ISSN: 0974-5343
IJMST (2021), 10(1):1-7

DOI: http://doi.org/10.5281/zenodo.4767293

Table 2: Comparison of oxidative stress parameters between NSTEMI and STEMI variants during acute myocardial infarction

Parameter	NSTEMI (Mean±SD)	STEMI (Mean±SD)	p value (Mean±SD)
Plasma Catalase (KU/L)	82.461 ± 34.53	56.384 ± 15.518 ***	P < 0.001, P = 0.0009
Plasma GSH (mg/dL)	0.17 ± 0.06	0.117 ± 0.04	P < 0.05, P = 0.012
Plasma SOD (U/L)	156.038 ± 14.373	145.769 ± 17.875 *	P < 0.05, P = 0.026
Plasma TBARS (µmol/L)	0.645 ± 0.262	0.862 ± 0.258 ns	P > 0.05, P = 0.053
Protein carbonyls (nmol/mg protein)	0.322± 0.172	0.414 ± 0.198	P > 0.05, P = 0.185

From table 2 it was observed that plasma catalase, GSH and SOD had decreased activity in STEMI group compared to NSTEMI significantly. The plasma TBARS and protein carbonyls did not alter significantly between NSTEMI and STEMI.

Statistics

Microsoft excel was used for statistical analysis. The sample size was 26 per each group. The average values of oxidative stress parameters were represented as Mean ± SD. For comparison between groups students t test was done with P value less than 0.05 (p<.05) considered as significant. The study wasdesigned as a comparative analysis of oxidative stress parameters between atherosclerotic disease high risk score category and low risk score category and between NSTEMI and STEMI variants in ECG during myocardial infarction.

Results

Table 1 is the compilation of data of oxidative stress parameters assessed between ASCVD risk score less than 7.5% and risk score greater than 7.5%. From the data it was understood that the antioxidant parameters such as catalase and glutathione (GSH) was significantly lowered in ASCVD risk score greater than 7.5% group compared to low ASCVD risk score group. It was noteworthy that another antioxidant enzyme, Superoxide dismutase (SOD) showed no significance between ASCVD low risk and high risk score groups. The pro oxidant parameters assessed were TBARS and protein carbonyls and found to be not significant between ASCVD low and high risk groups. Hence it can be inferred that antioxidant molecules such as GSH and catalase enzyme are early indicators of coronary artery diseases leading to acute myocardial infarction (AMI).

Table 2 is the compilation of data of oxidative stress parameters assessed between NSTEMI and STEMI variants of myocardial infarction. From the data it was observed that blood plasma had decreased catalase activity in STEMI compared to NSTEMI group with p value less than 0.01 and the same pattern of

IJMST

ISSN: 0974-5343
IJMST (2021), 10(1):1-7

DOI: http://doi.org/10.5281/zenodo.4767293

significance was observed for GSH and SOD with p value less than 0.05.

The pro oxidant parameters, TBARS and protein carbonyls did not show any significant difference between NSTEMI and STEMI group. Hence it can be inferred that the STEMI had compromised antioxidant defence compared to NSTEMI. Even with lowered antioxidant activity in STEMI, the TBARS levels were not altered which could be attributed to the efficiency of residual catalytic efficiency of SOD and catalase to neutralise the free radicals preventing lipid peroxidation.

The protein carbonyl content was assessed by Western blot by oxy blot assay. Figure 1 represented the Western blotanalysis of oxidised protein (Oxy blot assay). It was revealed that there was no obvious difference in band intensity between control, NSTEMI and STEMI group. This could indicate that the oxidative modification of protein did not influence myocardial infarction condition.

Western blot for detection of oxidised protein

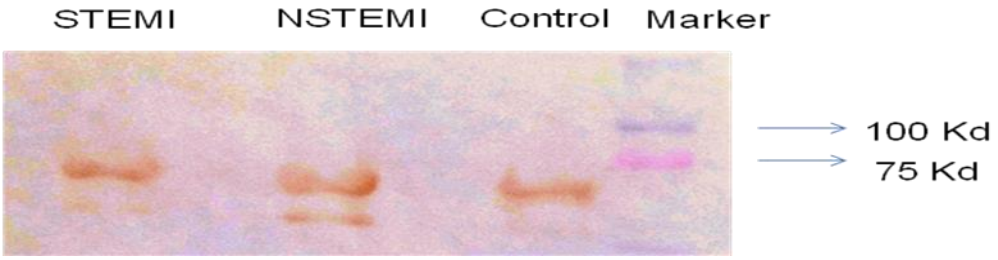

Figure - 1

Lane 1 was indicative of STEMI, Lane 2, NSTEMI, Lane 3, control, Lane 4, protein marker.
The oxy blot assay represented as Western blot detects the protein carbonyls. It was observed that protein carbonyls did not alter significantly between control, NSTEMI and STEMI plasma samples.

The Western blot indicated that the band intensity did not alter drastically between control, STEMI and NSTEMI group. It was observed that protein carbonylation detected in blood plasma did not influence the severity of myocardial infarction in the context of STEMI and NSTEMI.

Discussion

In the present study we analysed the oxidative stress parameters during acute myocardial infarction (AMI) in NSTEMI and STEMI patients. The study observed that there was reduction in antioxidant enzymes such as superoxide dismutase and catalase during AMI and marginal elevation though not significant in pro oxidant molecules such as thiobarbituric acid reacting substances (TBARS). The lowered catalase activity observed in both plasma and erythrocyte components in blood indicated that H_2O_2 accumulated in blood may trigger the oxidative stress signalling associated with coronary artery disease. The attenuated SOD activity during AMI indicated that superoxide anions would be poorly neutralised and damage the myocardium (16, 17). We also categorised the AMI into NSTEMI and STEMI groups based on variations in ECG pattern and observed that the antioxidant enzymes such as catalase and SOD were found to be decreased

IJMST

ISSN: 0974-5343
IJMST (2021), 10(1):1-7

DOI: http://doi.org/10.5281/zenodo.4767293

significantly in ST segment elevated MI variants (STEMI) compared to non ST segment variants (NSTEMI). The decrease was more evident in catalase activity during STEMI compared to other antioxidants such as SOD and GSH. SOD is known to prevent atherogenesis and associated cellular responses such as apoptosis, hypertrophy and cell migration. Elevated SOD activity confers protection against acute or chronic oxidative injury including atherosclerosis. Further SOD activity was reported to decrease with coronary artery disease progression. In a study it was reported that there was drop in erythrocyte catalase activity during myocardial ischemia reperfusion correlated with hyper homocysteinaemia (18). The present study also assessed the oxidative stress status in ASCVD high and low risk score groups with cut off as 7.5%. The study observed that antioxidant parameters such as glutathione (GSH) level and catalase activity decreased significantly in ASCVD high risk group compared to low risk group. The other antioxidant enzyme, SOD did not show any difference in activity between ASCVD low and high risk group. The pro oxidant parameter TBARS also was found to be non significant between ASCVD risk groups. The protein carbonyl content, another proxidant also was found to be not changed significantly between the study groups. This indicated that both catalase and GSH could be better oxidative stress indicators for possible coronary artery occlusions manifested as acute myocardial infarction. Besides, it can be inferred that the residual antioxidant enzyme activity is suffice enough to neutralise the proxidant free radicals such as lipid peroxides, superoxide anion, peroxides and protein carbonyls during myocardial infarction.

Conclusion

The study concluded that oxidative stress parameters such as catalase and glutathione are good indicators for acute myocardial infarction as indicated from their lowered activity in high risk ASCVD risk score group compared to lower risk group. The differential catalase and superoxide dismutase activity between NSTEMI and STEMI variants of myocardial infarction could be used to assess the severity of coronary blood vessel occlusion along with other conventional cardiac biomarkers such as troponin T and creatinine kinase (CK-MB). Further, the pro oxidant levels were not significantly altered between any study groups in spite of lowered antioxidant status.

Acknowledgement and funding:The financial support from Jubilee Centre for Medical Research is gratefully acknowledged.

Conflict of Interest: The authors declared that there is no conflict of interests regarding the publication of this manuscript.

References

[1] Prabhakaran D, Jeemon P, Roy A.(2016) Cardiovascular diseases in India: current epidemiology and future directions. Circulation.133(16),1605-20.

[2] Sanchis-Gomar F, Perez-Quilis C, Leischik R, Lucia A.(2016) Epidemiology of coronary heart disease and acute coronary syndrome. Annals of translational medicine.4(13).

[3] Manfredonia L, Lanza GA, Crudo F, Lamendola P, Graziani F, Villano A, Locorotondo G, Melita V, Mencarelli E, Pennestrì F, Lombardo A.(2019) Diagnostic role of echocardiography in patients admitted to the emergency room with suspect no-ST-segment elevation acute myocardial infarction. Eur Rev Med Pharmacol Sci.23(2),826-32.

IJMST

ISSN: 0974-5343
IJMST (2021), 10(1):1-7

DOI: http://doi.org/10.5281/zenodo.4767293

[4] Harrington DH, Stueben F, Lenahan CM.(2019) ST-Elevation Myocardial Infarction and Non-ST-Elevation Myocardial Infarction: Medical and Surgical Interventions. Critical care nursing clinics of North America.31(1),49-64.

[5] Kurian GA, Rajagopal R, Vedantham S, Rajesh M.(2016) The role of oxidative stress in myocardial ischemia and reperfusion injury and remodeling: revisited. Oxidative Medicine and Cellular Longevity.;2016.

[6] Gupta S, Sodhi S, Mahajan V.(2009) Correlation of antioxidants with lipid peroxidation and lipid profile in patients suffering from coronary artery disease. Expert opinion on therapeutic targets.13(8),889-94.

[7] Serdar Z, Serdar A, Altin A, Eryilmaz U, Albayrak S.(2007) The relation between oxidant and antioxidant parameters and severity of acute coronary syndromes. Acta cardiologica.62(4),373-80.

[8] Bagatini MD, Martins CC, Battisti V, Gasparetto D, Da Rosa CS, Spanevello RM, Ahmed M, Schmatz R, Schetinger MR, Morsch VM.(2011) Oxidative stress versus antioxidant defenses in patients with acute myocardial infarction. Heart and vessels.26(1),55-63.

[9] Filippo CD, Cuzzocrea S, Rossi F, Marfella R, D'Amico M.(2006) Oxidative stress as the leading cause of acute myocardial infarction in diabetics. Cardiovascular drug reviews. 24(2),77-87.

[10] Yang X, Li J, Hu D, Chen J, Li Y, Huang J, Liu X, Liu F, Cao J, Shen C, Yu L. (2016) Predicting the 10-year risks of atherosclerotic cardiovascular disease in Chinese population: the China-PAR Project (Prediction for ASCVD Risk in China). Circulation134(19),1430-40.

[11] Yagi K. Simple assay for the level of total lipid peroxides in serum or plasma. (1998) InFree radical and antioxidant protocols 101-106. Humana Press.12. Bruno S, Ronda L, Paredi G, Bettati S, Mozzarelli A. (2018) Protein carbonylation detection methods: A comparison. Data in brief. 19,2215-20.

[12] Levine RL, Garland D, Oliver CN, Amici A, Climent I, Lenz AG, Ahn BW, Shaltiel S, Stadtman ER. (1990) Determination of carbonyl content in oxidatively modified proteins. Methods in enzymology. 186,464-78.

[13] Hadwan MH, Abed HN. (2016) Data supporting the spectrophotometric method for the estimation of catalase activity. Data in brief. 6,194-9.

[14] Marklund S, Marklund G. (1974) Involvement of the superoxide anion radical in the autoxidation of pyrogallol and a convenient assay for superoxide dismutase. European journal of biochemistry 47(3),469-74.

[15] Rahman I, Kode A, Biswas SK. (2006) Assay for quantitative determination of glutathione and glutathione disulfide levels using enzymatic recycling method. Nature protocols. 1(6),3159.

[16] Serdar Z, Serdar A, Altin A, Eryilmaz U, Albayrak S.(2007) The relation between oxidant and antioxidant parameters and severity of acute coronary syndromes. Acta cardiologica. 62(4),373-80.

[17] Bagatini MD, Martins CC, Battisti V, Gasparetto D, Da Rosa CS, Spanevello RM, Ahmed M, Schmatz R, Schetinger MR, Morsch VM. (2011) Oxidative stress versus antioxidant defenses in patients with acute myocardial infarction. Heart and vessels. 26(1),55-63.

[18] Noichri Y, Chalghoum A, Chkioua L, Baudin B, Ernez S, Ferchichi S, Miled A (2013) Low erythrocyte catalase enzyme activity is correlated with high serum total homocysteine levels in Tunisian patients with acute myocardial infarction. Diagnostic Pathology 8(1);68.